IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re application of: Cheng J. Cao et al.
Serial No. 10/631,224
Filed: July 28, 2003
For: METHODS FOR DETERMINING THE PRESENCE OF STAPHYLOCOCCAL ENTEROTOXIN A GENE IN A SAMPLE
Attorney Docket No.: DAM 581-02

Group Art Unit: 1645
Examiner: Khatol S. Shahnan Shah

Honorable Commissioner of Patents and Trademarks
Alexandria, VA 22313-1450

APPEAL BRIEF UNDER 37 CFR § 41.37

1. REAL PARTY IN INTEREST

The Real Party in Interest for the above identified application is the United States of America, as represented by the Secretary of the Army. The United States of America, as represented by the Secretary of the Army, is the Real Party in Interest by assignment from all the inventors.

2. RELATED APPEALS AND INTERFERENCES

Neither the appellant, the appellant's legal representative, nor the assignee know of any other appeals or interferences which will have any bearing on the Board's decision in the pending appeal.

3. STATUS OF THE CLAIMS

Claims 15, 23 and 24 are pending in the application and have been finally rejected and are on appeal. Claims 1-14 and 16-22 have previously been canceled.

4. STATUS OF AMENDMENTS

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No amendments have been filed subsequent to the response filed September 23, 2008, in response to the examiner's non-final Office Action which was mailed on April 24, 2008. A final rejection was mailed on January 26, 2009, and a Notice of Appeal was filed on May 20, 2009, in response thereto. It should be noted that in response to an indication of allowable subject matter for dependent Claim 16 earlier in prosecution, Applicant amended Claim 15 to include the limitations of Claim 16 to place Claim 15 in condition for allowance. However, newly amended Claim 15 was subsequently rejected in a later Office Action.

5. SUMMARY OF CLAIMED SUBJECT MATTER

The present invention pertains to a method of determining the presence of staphylococcal enterotoxin A gene in a sample. More particularly, to assays comprising nucleic acid probes and primers, and methods of using the same for detecting nucleic acids associated with encoding or expressing staphylococcal enterotoxin A in a sample. Specifically, independent Claim 15 now recites "A method of determining the presence of staphylococcal enterotoxin A gene in a sample, comprising: contacting a target nucleic acid sequence which comprises a portion of the *S. aureus ent A* gene encoding staphylococcal enterotoxin A, with polymerase chain reaction reagents specific for the target nucleic acid sequence, the polymerase chain reaction reagents including a primer selected from the group consisting of a forward primer having a specific sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4 and combinations thereof, and a reverse primer having a specific sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6 and combinations thereof, a polymerase enzyme, and a nucleic acid probe, wherein the nucleic acid probe further comprises: a nucleic acid sequence that hybridizes to a portion of the target nucleic acid sequence wherein the portion is unique to the nucleic acid

encoding staphylococcal enterotoxin A, and wherein the nucleic acid sequence of the nucleic acid probe is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and combinations thereof; a reporter attached to a 5' end of the nucleic acid probe, said reporter capable of emitting a detectable signal; a quencher attached to a 3' end of the nucleic acid probe capable of substantially quenching the reporter and prevent emission of the detectable signal, when the nucleic acid probe is intact, wherein the reporter becomes substantially unquenched when the nucleic acid probe is cleaved by the polymerase enzyme during amplification of the target nucleic acid sequence; amplifying the target nucleic acid sequence by thermal cycling, wherein the thermal cycling will amplify the target nucleic acid sequence; and measuring the level of fluorescence in the sample subsequent to thermal cycling, and further wherein the level of detectable signal is correlated to an amount of the nucleic acid encoding staphylococcal enterotoxin A in the sample, thereby quantitatively detecting the nucleic acid encoding staphylococcal enterotoxin A in the sample." Claims 23 and 24 are directly dependent from Claim 15 and are further limiting thereto.

Figure 1 depicts a target gene region [SEQ ID NO: 7] of a target nucleic acid sequence of the Staphylococcal *aureus* entA gene [SEQ ID NO: 8] that encodes staphylococcal enterotoxin A wherein sequences indicated by a single line represent preferred forward primers [SEQ ID NO: 3] and [SEQ ID NO: 4], respectively, wherein sequences indicated by double lines represent nucleic acid complements of preferred reverse primers [SEQ ID NO: 5] and [SEQ ID NO: 6], respectively, and wherein sequences indicated by dotted lines represent preferred nucleic acid probes [SEQ ID NO: 1] and SEQ ID NO: 2], respectively, in accordance with the present invention. Also see specification page 13, line 10, through page 23, line 13, for description of

the assay method including the specific embodiment of Claim 15. See the specification page 23, line 15, through page 26, line 21, for description of the "reporters" and "quenchers" of Claim 23 and 24, respectively.

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

(a) Whether or not Claims 15, 23 and 24, should be rejected under 35 U.S.C. §103(a) as unpatentable over Letertre et al. (Molecular and Cellular Probes, vol. 17, pp. 139-147, 2003) in light of O'Connell et al. (23rd Army Science Conference, December 2002), further in view of Borst et al. (Infection and Immunity vol. 61, no.12, pp. 5421-5425, 1993 and sequence alignment # STAENAB) and further in view of Padmapriya et al. (US 2005/0233345A1 and sequence alignment #AED45640). Claims 23 and 24 are directly dependent from Claim 15. Therefore, if Claim 15 is allowed Claims 23 and 24 should also be in condition for allowance.

7. ARGUMENT

(a) Whether or not Claims 15, 23 and 24, are unpatentable under 35 U.S.C. §103(a) over Letertre et al. (Molecular and Cellular Probes, vol. 17, pp. 139-147, 2003) in light of O'Connell et al. (23rd Army Science Conference, December 2002), further in view of Borst et al. (Infection and Immunity vol. 61, no.12, pp. 5421-5425, 1993 and sequence alignment # STAENAB) and further in view of Padmapriya et al. (US 2005/0233345A1 and sequence alignment #AED45640).

Claims 15, 23 and 24 were rejected under 35 U.S.C. §103(a) as being unpatentable over the publications of Letertre et al. in light of O'Connell et al., further in view of Borst et al., and further in view of Padmapriya et al. Letertre and O'Connell were relied on to teach the method steps of real-time fluorescence PCR, while Borst was relied on to teach primers "100% identical

to SEQ ID NO: 3, 4 and SEQ ID NO: 5, 6 (see page 5423 and sequence alignment #STAENAB for SEQ ID NO: 3 and NO: 5.) It was also asserted Borst teaches "primers and probes in the size of 20 to 21 bases in length from an entire sea gene having a length of 1443 bases (see Borst et al. pages 5422-5423 and sequence alignments for SEQ ID # 3, 4 and SEQ ID #5, 6)." Finally, it was asserted that Padmapriya teaches "primers of size of 20 or more nucleotides 100% identical to SEQ ID NO:1 and 2 which target *Staphylococcus* enterotoxin A gene and used in detecting Staphylococcus aureus causing food poisoning (see abstract, claims and sequence alignment # AED45640)." It is respectfully submitted that this argument is untenable and should be withdrawn because Borst and Padmapriya do not actually teach primers identical to those disclosed and claimed by applicant, and therefore, the combination of Letertre, O'Connell, Borst and Padmapriya does not teach the primers and probes claimed by applicant in the claimed method.

More specifically, the primer sequences taught in Borst are simply not designed for real-time PCR and differ from applicant's primers in several respects. First, the primer sequences taught in Borst amplify a sequence of 272 base pairs in length. On the other hand, target sequences that are optimal for real-time fluorogenic PCR are in the range of 50-150 base pairs in length. Accordingly, applicant's primers amplify sequences of 101 and 99 base pairs in length. In addition, Borst simply does not teach primers with sequences identical to those claimed by applicant. Borst teaches an upstream primer of 5' AGCATACTGCAAGTGAAGTTG 3' and a downstream primer of 5' TTGTTGTCAACGTTAGGG 3'. These sequences are not matches with the primer sequences claimed by appellant in Claim 15. It is believed that any match found to appellant's sequences

probably resulted from the fact that the entire sequence of Accession L22565 was referred to in the Borst publication. L22565 comprises the entire sequence of accession for the upstream region of the *sea* gene. Therefore, it may include as part of the entire sequence those portions identified by applicant as SEQ ID NO: 3 and SEQ ID NO: 5, but that does not constitute a teaching of those specific primer sequences which are only a small part (20 or 21 bases) of the L22565 Accession. Furthermore, the majority of SEQ IDs which are claimed and disclosed by appellant are not included in the L22565 Accession at all since it does not include the entire *sea* gene. Appellant has claimed specific primers and probes of about 20 or 21 bases in length from an entire *sea* gene having a length of 1443 bases.

It is also important to note that while Claim 15 uses the open transitional term "comprising" which is open-ended with respect to the overall method steps, it also includes as limitations "a primer selected from the group *consisting of* a forward primer having a specific sequence selected from the group *consisting of* SEQ ID NO: 3, SEQ ID NO: 4 and combinations thereof, and a reverse primer having a specific sequence selected from the group *consisting of* SEQ ID NO: 5, SEQ ID NO: 6 and combinations thereof" and "wherein the nucleic acid sequence of the nucleic acid probe is selected from the group *consisting of* SEQ ID NO: 1, SEQ ID NO: 2." Thus, the specific primers and probes used in appellant's claimed method are specifically limited to the sequence listings recited by appellant in Claim 15.

In the final Office Action, the examiner incorrectly asserts that the claim language "a primer selected from the group consisting of a forward primer having a specific sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4 and combinations thereof, and a reverse primer having a specific sequence selected from the group consisting of SEQ ID

NO: 5, SEQ ID NO: 6 and combinations thereof..." and "..wherein the nucleic acid sequence of the nucleic acid probe is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and combinations thereof..." is open language because the term "having" is used. It is respectfully submitted that this is incorrect because with respect to the primers the claim expressly recites "a *specific sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4*" and "*a specific sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6* and combinations thereof..." and with respect to the probe "wherein the nucleic acid sequence of the nucleic acid probe *is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and combinations thereof...*" It should be clear that Claim 15 is limited to the specific sequences recited in the claim by the language "selected from the group *consisting of.*"

Furthermore, the Borst sequences violate several of the primer set design guidelines that must be followed to obtain a set of primer and probe oligonucleotide sequences that will perform optimally in real-time fluorogenic PCR. Specifically, the two primers described in Borst are not suitable for use in identifying the *entA* gene by real-time fluorogenic PCR for several reasons, including: (a) the amplicon being of 272 base pairs while real-time fluorogenic PCR optimally requires 50-150 base pairs; (b) Borst does not teach the melting temperature of their primer pair, and primer pairs designed for optimal performance in real-time fluorogenic PCR have melting temperatures between 58 and 60 degrees C; and (c) the downstream primer sequence taught in Borst violates the guideline for primer selection that no more than two of the five bases at the 3' end of a primer be either G or C (Borst teaches a downstream primer with three G/C bases among the five bases at the 3' end of the primer).

Moreover, only one of the primers taught by Borst (that appearing in the text on p. 5423) binds a sequence inside the open reading frame encoding the SEA protein. The other primer binds a sequence upstream of the promoter for the *entA* gene. The use of this primer pair will fail to detect the gene if the gene has been excised from its native sequence and cloned behind a promoter that has been optimized for expression of the gene in another organism (as may be the case if the gene is used to create a genetically engineered biological weapon). Therefore, in addition to not being suitable for real-time PCR, the primers described on pages 5422 and 5423 do not constitute a functioning assay directed specifically and solely at sequences that encode staphylococcal enterotoxin A.

Furthermore, the primer sequences taught in Padmapriya are not the same as applicant's claimed SEQ ID No: 1 and SEQ ID NO: 2. They simply are not 100% identical. Close inspection will reveal that Padmapriya's forward primer overlaps an internal portion of applicant's *probe* sequence SEQ ID: NO 1, not a primer sequence. Likewise, a close inspection of Padmapriya's reverse primer sequence reveals that it is not identical to or overlapping any of applicant's primer or probe sequences SEQ ID NO: 1 through 6.

In addition, the size of the amplicon resulting from the use of Padmapriya's primers is 301 bases in length, which is more than double the optimal size for real-time PCR, and therefore, can not be combined with the teachings of Borst et al. or Letertre et al. to make optimal real-time fluorescent PCR assays. Finally, the combination of prior art cited does not specify probe sequences at all.

Appellant readily acknowledges that the general practice of real-time fluorogenic PCR is well established among molecular biologists. However, the assay that is the subject of

Appellant's claims is a specific, non-obvious application of the technique designed to detect the presence of nucleic acids encoding the *entA* gene in a sample. The unique, non-obvious feature of the assay is the sequences selected to form the primer and probe oligonucleotides one would use in the performance of the assay.

The examiner broadly asserts in the final Office Action that the "choice of primers" is well established among molecular biologists. Appellant disagrees. Furthermore, this position is not in compliance with patent examination procedures appellant has experienced in previous molecular biology patent applications.

Appellant reiterates that the selection of specific sequences for PCR primers and probes is not obvious. A researcher should not expect optimal results, or indeed, any results at all, by merely selecting random sequences 20 bases in length to serve as forward and reverse primers. All workers who design PCR assays must obtain several primer pairs and test them empirically to determine which pairs work well (if any at all) under the desired reaction conditions. While Letertre et al. do describe PCR assays that detect the *entA* gene, the assays described are up to 25-fold less sensitive than applicant's assays and are clearly not identical.

In a previous Office Action the examiner noted that one can not show non-obviousness by attacking references individually where the rejection is based on the combination of references. However, where individual references are relied on as teaching particular elements/limitations in applicant's claims, applicant is permitted to argue that those individual references do not actually teach what the examiner is asserting they teach, and therefore, that a *prima facie* case of obviousness has not been established. Such is the case here, where applicant

is arguing that Borst and Padmapriya do not, in fact, teach primers and probes which are 100% identical to those of SEQ ID NOs: 1-6 as claimed in Claim 15.

In summary, Claims 15, 23 and 24 are on appeal and based on the foregoing remarks and arguments should be considered in condition for allowance. Accordingly, it is respectfully submitted that the pending claims are patentable and in condition for allowance. Early reconsideration and withdrawal of the rejections is earnestly solicited, as is allowance of the claimed subject matter.

Respectfully submitted,

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DATE

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8. APPENDIX OF THE CLAIMS ON APPEAL

Claim 15. A method of determining the presence of staphylococcal enterotoxin A gene in a sample, comprising:

contacting a target nucleic acid sequence which comprises a portion of the *S. aureus ent A* gene encoding staphylococcal enterotoxin A, with polymerase chain reaction reagents specific for the target nucleic acid sequence, the polymerase chain reaction reagents including a primer selected from the group consisting of a forward primer having a specific sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4 and combinations thereof, and a reverse primer having a specific sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6 and combinations thereof, a polymerase enzyme, and a nucleic acid probe, wherein the nucleic acid probe further comprises:

a nucleic acid sequence that hybridizes to a portion of the target nucleic acid sequence wherein the portion is unique to the nucleic acid encoding staphylococcal enterotoxin A, and wherein the nucleic acid sequence of the nucleic acid probe is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and combinations thereof;

a reporter attached to a 5' end of the nucleic acid probe, said reporter capable of emitting a detectable signal;

a quencher attached to a 3' end of the nucleic acid probe capable of substantially quenching the reporter and prevent emission of the detectable signal, when the nucleic acid probe is intact, wherein the reporter becomes substantially unquenched when the nucleic acid

probe is cleaved by the polymerase enzyme during amplification of the target nucleic acid sequence;

amplifying the target nucleic acid sequence by thermal cycling, wherein the thermal cycling will amplify the target nucleic acid sequence; and

measuring the level of fluorescence in the sample subsequent to thermal cycling, and further wherein the level of detectable signal is correlated to an amount of the nucleic acid encoding staphylococcal enterotoxin A in the sample, thereby quantitatively detecting the nucleic acid encoding staphylococcal enterotoxin A in the sample.

Claim 23. The method of claim 15, wherein the reporter is selected from the group consisting of 1-dimethylaminonaphthyl-5 sulfonate, 1-anilino-8-naphthalene sulfonate, 2-p-touidinyl-6-naphthalene sulfonate, 3-phenyl-7-isocyanatocoumarin, 9-isothiocyanatocacridine, N-(p-(2-benzoxazolyl)phenyl)maleimide, benzoxadiazoles, stilbenes, pyrenes, 6-carboxyfluorescein, tetrachloro-6-carboxyfluorescein, 2,7-dimethoxy-4,5-dichloro-6-carboxyfluorescein, hexachloro-6-carboxyfluorescein, 5-carboxyfluorescein, 6-carboxy-2',4,7,7'-tetrachlorofluorescein, carboxy-X-rhodamine and 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein.

Claim 24. The method of claim 15, wherein the quencher is selected from the group consisting of 6-carboxytetramethylrhodamine, tetramethylrhodamine and 4-(4-dimethylaminophenylazo) benzoic acid.

9. EVIDENCE APPENDIX

No evidence pursuant to 37 CFR §§1.130, 1.131, or 1.132 has been submitted in the present application.

10. RELATED PROCEEDINGS APPENDIX

No other appeals, interferences or prior judicial proceeding exists for the present application, therefore, no copies of prior decisions rendered by a Court or the Board is available.

P.017